ISOLATION AND CHARACTERIZATION OFCONCANAVALIN A FROM JACK BEAN (*CANAVALIA ENSIFORMIS*) SEED

A THESIS SUBMITTED FORPARTIAL FULFILLMENT OF THE

MASTER OF SCIENCE DEGREE
IN
LIFE SCIENCE

SUBMITTED TO

NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

BY
SANGEETA MINZ
ROLL NO. 410LS2058
UNDER THE SUPERVISION OF
ASSISTANT PROF. DR. SUJIT KUMAR BHUTIA

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY
ROURKELA-769008, ODISHA, INDIA

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY,
ROURKELA-769008.

.....

Dr. Sujit Kumar Bhutia

Assistant Professor.

Department of Life Science

National Institute of Technology

Rourkela – 769008, Odisha, India
Email: sujitb@nitrkl.ac.in

CERTIFICATE

This is to certify that the thesis entitled "**Isolation And Characterization of Concanavalin A from Jack bean (*Canavalia ensiformis*) seed**" which is being submitted by **Miss Sangeeta Minz**, Roll No. **410LS2058**, for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

Dr. Sujit K Bhutia.
Date: 03/05/2012

DECLARATION

I, Sangeeta Minz, hereby declare that, the project report entitled **"Isolation andcharacterization of Concanavalin A from jack bean *(Canavalia ensiformis)* seed"** is the original work carried out by me under the supervision of Dr. Sujit kumar Bhutia, Assistant Professor Department of Life Science , National Institute of Technology Rourkela (NITR), Rourkela and to the best of my knowledge and belief the present work or any other part thereof has not been presented to any other University or Institution for the award of any other degree.

Sangeeta Minz

ACKNOWLEDGEMENTS

I wish to express my deepest sense of gratitude to my supervisor Dr. SujitkumarBhutia, Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela for his valuable guidance, assistance and time to time inspiration throughout my project.

I am very much grateful to Dr. Samir kumar Patra, H.O.D of life science department, National Institute of Technology, Rourkela for providing excellent facilities in the Institute for carrying out research.

I would like to take the opportunity to acknowledge quite explicitly with gratitude my debt to all the Professors and Staff, Department of Life Science, National Institute of Technology, Rourkela for his encouragement and valuable suggestions during my project work.

My heartfelt thanks to all the PhD scholars and my friends for their inspiration and support throughout my project work

Sangeeta Minz

CONTENTS

ABSTRACT

The study was done to isolate Concanavalin A from jack bean *(canavalia ensiformis).*The protein was purified by using affinity chromatography. The activity of the lectins was determined by haemagglutinin assay and the purity of the protein was tested by Sodium dodecyl sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The activity of lectins is quantified by their ability to agglutinate erythrocyte. The eluted protein exhibited agglutinating activity when reacted against different types of fresh erythrocytes. In SDS-PAGE in affinity bands was observed of 104 kDa. It is a homotetramer, each subunit bind with metallic atom (Mn^{2+} and Ca^{2+}).

(Key word- Concanavalin A, affinity chromatography, SDS PAGE)

INTRODUCTION

1. INTRODUCTION

Plant lectins are sugar- binding proteins or carbohydrate binding proteins, which are proteins containing sugar chains or residues (Goldstein *et al,* 1980). Most of lectins are multivalent and capable of agglutinating cells. The word "Lectin" is derived from the Latin word "*Legere"*, means "to select".'Peter Hermann Stillmark' (1888) had given the concept of lectins. He isolated lectins from seed of the "Castor Plant" (*Ricinus communis).* The first lectin purified on large scale and used on a commercial used was Concanavalin A.

Lectins are used in medicine and medical research. Purified lectins are used in a clinical as for blood typing. Lectins have been widely used as the molecular basis of protein recognizes carbohydrates. Affinity chromatography, blotting, affinity electrophoresis and affinityimmunoelectrophroesis these are used for purifying concanavalin A.

1.1 Biological function

Lectins are ubiquitous and it found in animals, plants and microorganism.

Function in animals - The chief function of lectins in animals is cell adhesion to glycoprotein synthesis. It maintains the level of protein in the blood. Some lectins found on surface of liver cells in mammals for recognized galactose residues. In the immune system lectins recognized carbohydrates and found on pathogens, or inaccessible on host cells.

Function in plants:-Lectins are found in seed of leguminous plant and it present in other part of the plant body, like leaves, stem and bark (Van Rhijn P *et al,* 1998). Some function of plant body occurs naturally (Skvortso I M *et al,* 1998) such as –

i) Storage of protein in seed.

ii) Maintained the seed dormancy.

iii) Protect from pathogens.

iv)Transportations of carbohydrate.

v) Cell walls are elongated.

Lectins are used in transgenic expression (Rao K V *et al*,1998). For example soybean agglutinin gene changes the nodulation factor of Bradyrhizobium.

1.2 Classification

On the basis of structural point of view mature Lectins are subdivided of following:

i) Merolectins.
ii) Hololectins.
iii) Chimerolectins.
iv) Superlectins.

Merolectins- These types of lectins are single domain of carbohydrate-binding. Due to monovalent nature they don't precipitate glycocojugates and agglutinated cells. (Van Parijs *et al*,1991)

e.g - Chitin (*hevea brasiliensis*)

Hololectins- These are carbohydrate-binding protein with two or more domain. It is homogenous in nature and binds with the similar sugar(Shu-Ye Jiang,2010). So that they agglutininate with the cell or precipitate glycoconjugates.

Chimerolectins- They are multivalent carbohydrate-binding domain. Some unrelated domains are biological activity and function of carbohydrate binding domain and these domains are independent sometimes. Chimerolectins act as Merolectins.

Superlectins-It contains at least two carbohydrate binding domain. They are structurally unrelated sugar; special group of chimerolectin consists of two tandemly arrayed and different functionally (Van Damme *et al*, 1996).

According to their molecular weight three type of lectins are present(Sharon and Lis, 1998)such as-

i) Simple lectins.
ii) Mosaic(multi domain) lectins.
iii) Macromolecular assemblies.

Simple lectins-The molecular weight of simple lectins usually below 40 kDa. It contains small subuits and additional domain for carbohydrate binding site(s). Maximum number of plant lectinsaregalectins(s-lectins).

Mosaic(multidomain)lectins-It is monovalent and consists of subunit with high range of molecular weight. It contain many kinds of protein domain but only one can possesses a carbohydrate- binding site .These domain are diverse protein from different source, such as animal lectins of c-,l-,p- type and viral hemagglutinins.

Macromolecular assemblies- This type of lectins found in bacterial cell in the form of pili. It looks like filamentous heteropolymericorganelles and contain different type of helical subunits. (Ofek *et al*, 1990). That fimbrial composed up polymers subunits which has plays structural role. Only one minor subunit of fimbriae possesses a carbohydrate-binding site.

1.3 Plants lectins

Plant lectins are divided into seven families according to evolutionary and structurally related protein. These seven families included in three groups-

i) Legume lectins
ii) Monocot mannose- binding lectins
iii) Chitin-binding lectins

Legume lectins-

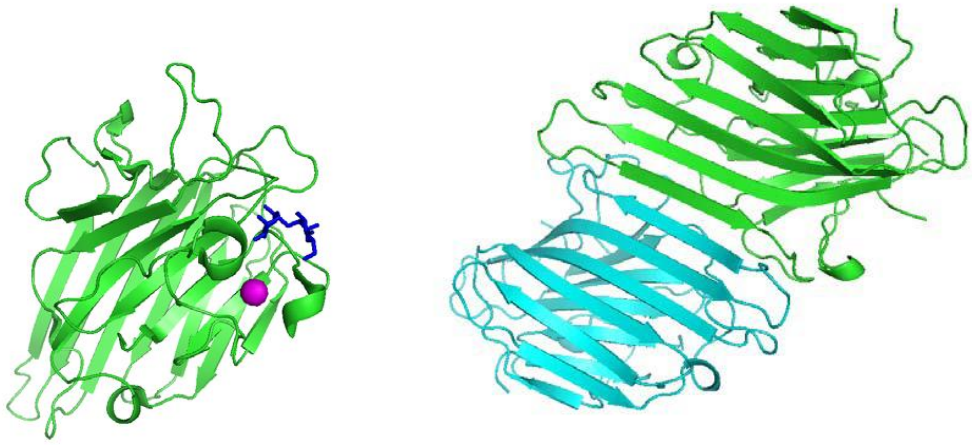
Legume lectins have achieved the successes in the field of bio-chemistry, physiology and cancer biology. First purified and crystallized lectins were Con A (Summer and Howell, 1938).

It has resolved the primary structure and there dimensional structure. Most of plants lectins are isolated from seeds.

Legume lectins are present in dimeric or tetrameric in structure, maximum number are single chain proteine.g.Con A. They are tightly bound with Ca^{2+} and Mn^{2+} ion and it is necessary for carbohydrate with lectins interaction.

Most of the lectins are similar with 3-dimensional structure. Monomer is built from seven beta sheets from front face and six beta sheetsforms from back site. They are inter-connected with each other by loop and flattened doom shaped structure is formed. It is known as jelly-roll-motif. Four loops found at the upper site dome and formed monosaccharide binding site.

Dimericlectins are divalent where astetramericlectins are tetravalent with four carbohydrate binding sites. 'canonicaldimer'formed form non covalent monomers (fig-1a) and back walls are formed teramer(fig-1b) .A part from carbohydrate-binding site it has bind site for hydrophobic molecules e.g. 1,8 anilinonaphthalenesulfonic,adenine, TNS etc (Robert and Goldstein, 1983).hydrophobic binding site and carbohydrate binding site are different with each other. Legume lectins are help in symbiosis of plant and micro-organism (Diaz *et al*, 1986).



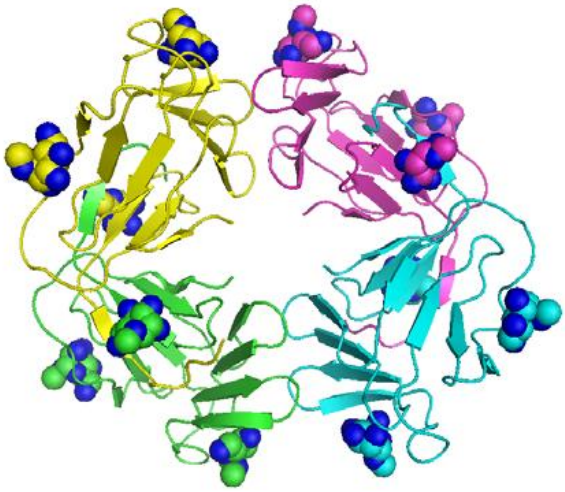
(a) (b)

fig-1(a)and(b)- Three dimenssion structureLegume lectins

Monocot mannose binding lectins

These lectins are include in mannose specific lectins and subgroups areknown as monocot families such as like Alliaceae, Amaryllidaceae, Araceae, Bromeliaceae, Liliaceae, and Orchidaceae.They are found in different tissue of leaves,bulb, tubers, flowers, root and even in necrar, rarely in seed.

First isolated lectins of this family is Galanthusnivalisagglutinin(GNA). From three-dimensional structure, it has beta-prism II fold. Monomers of this family contain three tandemly arrayed subdomains (I, II and III). Each of them contain four-stranded beta-sheets and it has a carbohydrate recognition domain (CRD). These three subdomains have local three –fold symmetry and form prism. From the connection of loop it has form 12 stranded beta-barrel, which is found at mannose-binding site. Its two dimers are tetramers and interaction of hydrophobic .So that GNA molecules is 12 mannose-binding sit(fig-2a) and with three putative carbohydrate –binding site(fig-2b).These lectins are protect from sucking insect (Rehbe *et al*, 1995) and invertebrates(Hilder *et al*, 1995).



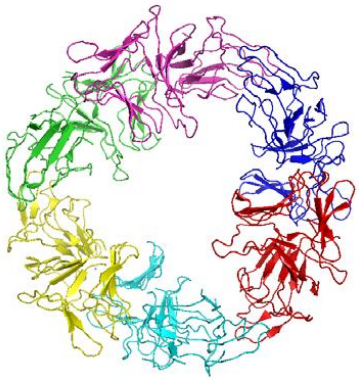


Fig-2(a) Fig-2(b)

Fig2(a) and(b) Monocot mannose binding lectins structure

Chitinous –binding lectins

This family formed from all proteins, which has contains at least one hevein domain (Raikhel *et al*, 1993). Hevein consists of 43 amino acid protein of latex of rubber tree. It possesses chitin –binding activity. From thislectins included families are Gramineae, Solanaceae, Phytolaccaceae,Urticacea, Viscaceae and Papavaracaceae. It is typically merolectin, with addition of chitin-binding antimicrobial peptides. It contains two short α-helices and amino acid residues, which was located at the N-terminal end of polypeptide chain. Antiparallel of β sheets strand arranged according to α-helix(fig-3).

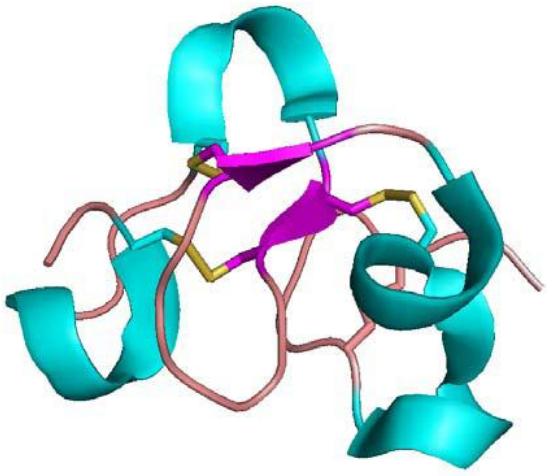


Fig- 3.Chitinous –binding lectins

REVIEW AND LITERATURE

3. REVIEW OF LITERATURE

Jack bean is a legume crop. Scientifically known as *canavalia ensiformis* .It is used for animal fodder and human nutrition. Jack bean was origin to the Central America and west-indies. Now it widely distributed in area of tropical and subtropical. This crop produces from united state, Brazil, Argentina, China and India. It was used for tropical soil reclamation efforts,avoid the wide range of soil acidity,salinity condition and capable of nitrogen fixation. Most of the times jack bean were free from pets and disease. Sometimes root rot by fungus *colletotrichumlindemuthianum*has found in some region. These legume plants are not cultivated for commercially because it can mildy toxic and copious consumption must be avoided. The main propose of jack bean is plant lectins, which is known as concanavalin A. This plant lectin usually helps in apoptosis.

3.1Classification of (*canavalia ensiformis)*

Kingdom- *Plantae*

Subkingdom *Tracheobionta*

Superdivision *Spermatophyta*

Division *Magnoliophyta*

Class *Magnoliopsida*

Subclass *Rosidae*

Order *Fabales*

Family *Fabaceae*

Genus *Canavalia*

Species *ensiformis*

Fig-4-: Jack bean plant

Fig-5-: Jack bean seed

3.2 Concanavalin A

Concanavalin A is a plant proteins called as lectins. It was the first legume lectin that was purified and crystallized from the seeds of Jackbean (*Canavalia ensiformis*). It was a single-cell protein with three dimensional structures. It binds with thenonreducing terminal α-D-mannosyl and α-D-glucosyl groups of sugars, glycoproteins, and glycolipids(Goldstein and Poretz, 1986). It is a metalloprotein containing Mn^{2+} ion and Ca^{2+}ion. Under the influence of pH, Con A can exist as a dimer or a tetramer(Loris *et al,* 1998). It can also exists as dimetallised dimer in two different forms i.e., in dimeric or tetramericforms(Brewer *et al,* 2007).

Characteristics of Concanavalin A

Concanavalin A consists of 237 amino acid residues and no cysteine residue (Olson and Liener 1967) **.** It is a single dimer, its molecular weight 53000(McKenzie *et al,* 1972) .Two metal ions are binds with Con A like Mn^{2+} and Ca^{2+}(Becker *et al,* 1975). Con A reacts with non-reducing α-D-glucose and α-D-mannose (Goldstein and Poretz 1986).

The active sitesof α-methyl-D-glucopyranoside act like competitive inhibitor in the stereochemical requirements (Smith andGoldstein 1967).

Concanavalin A is also a plant mitogen since it has the ability to activate mouseT-cell subsets that give rise to four T-cell populations such as precursor to suppressor T-cell, one subset of human suppressor T-cells sensitive to Con A(Powell and Leon 1970).The usefulness of ConA use in specific binding action with carbohydrate-containing receptors. It agglutinates red blood cells. (Clark and Denborough 1971).

Structure of Concanavalin A

Thelectinconcanavalin A structure is three-dimensional with tentative amino-acid sequence**(**Bruce A*et al,* 1972).It is a homotetramereach subunit(26.5kDa) containing 237 glycated amino acids that bind to metallic ion i.e., Ca^{2+} or Mn^{2+}(Min W *et al*, 1972). According to amino acid chain it has two anti-parallel beta sheets Monomer is built from seven beta sheet from fornt face and six beta sheet form from back site. They are inter-connected with each other by loop and flattened doom shaped structure is formed. It is known as" jelly-roll-motif". Four loops found at the upper site dome and formed monosaccharide binding site.The metal atoms bindswith sugar binding site. Its molecular weight is 104 to 112 kDa and isoelectric point is between 4.5 to5.5. A part from carbohydrate-binding site it has bind site for hydrophobic molecules.

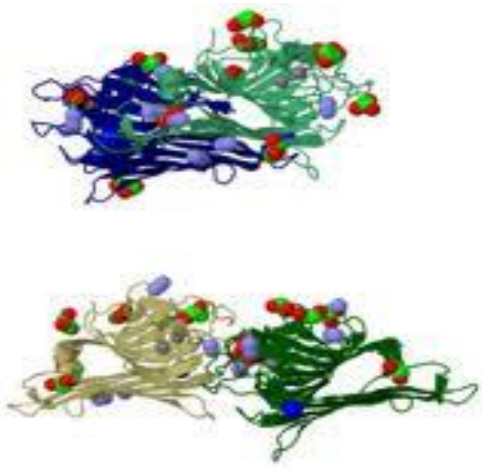


Fig-6:Homotetrameric structure of concanavalin A.

Biological activity of Concanavalin A

Concanavalin A was interact with receptor, which was contain mannose carbohydrate, insulin receptor the Immunoglobulin, blood group markers and carinoembryonary antigen. It was strongly agglutination in erythrocytes of irrespective of blood group and different type of cancerous cells (Betton GR 1976,Kakizoe T *et al*, 1980, Becker FF *et al*,1975). At 4^0c transfer of cells and trypsin-treated normal cells were not agglutinated. So that, temperature –sensitive step was involved in Concanavalin A-mediated agglutination(Inbar M *et al*,1971,Sela B *et al*,1971).

Agglutination of other cell types were like muscle cell(myocytes)(Gartner TK and Podleski TR 1975). B lymphocytes,through surface immunoglobulin adipocytes.(Cuatrecasas P 1973), human fetal(not adult) intestinal epithelial cell(Weiser MM 1972), fibroblast(Noonan KD and Burger MM 1973), and rat thymocytes(Capo C *et al* 1982)

Many micro-organism like E.coli(Ofek I *et al*, 1977), Bacillus subtilis and Dictyostellium(Doyle RJ, and Birdsell Dc 1972) were containing mannose residue and Concanavalin A was react with their surface of mannose residues.

Concanavalin A. was bound with the glycoprotein of cell membrane by ligands like alpha-methyl-D-mannoside. When antibody were added to Con A it forms antibody complex. Complex was not dissociated from membrane by alpha-methyle-D-mannoside. Antibody to Con A were completely suppress. It stimulated the lymphocytes and measured by tritiated thymidine. In migration inhibition, enhancement of plaque forming cell.

Application of concanavalin A

Concanavalin A. aggregation used to study for relationship between cell toxicity and aggregation process (Vetric.V *et al*,2010). It has antiproliferative effect on human cancer cells. Con A was induced apoptosis of cancer cells lacking function of p53. Again Con A lectins was induced cancer cell death through a mitochondria mediated autophagic pathway, where it induced human melanoma A375 cell in caspase-dependent manner(Liu B *et al*, 2009).

Concanavalin A was recently reported for therapeutic effect on hepatoma (liver cancer)(Lei HY, and Chang CP 2009). Concanavalin A used in solid phase of immobilized glycoenzymes, because they were difficult to immobilize by traditional covalent coupling.

It was used in immuno-affinoelectrophoresis to detect hormone associated variation in α_1 acid glycoprotein. (Christine wells *et al,* 1980).

OBJECTIVES

4. OBJECTIVES

- **Isolation and characterization of Concanavalin A from Jackbean seeds**
- **Measurement of protein concentration**
- **Haemagglutination assay**
- **SDS PAGE**

MATERIAL AND METHODS

5. MATERIALS AND METHODS

5.1 Sample collection: Dry Jack bean seed were collected from IIT KGP, Department of Bio-technology.

5.2 Chemical

Acrylamide, bisacrylamide,Sodium dodecyl sulphate (SDS), Ammonium persulphate (APS),N,N,N',N'-tetramethylenediamine (TEMED), β metacarpoethanol,Bovine serum albumin(BSA), Tris were purchased from Sigma Aldrich, USA.Sodium hydroxide (NaOH),Sodium carbonate (Na_2CO_3), Potassium sodium tartarate ($KN_aC_4H_4O_6$), glycine, Coppersulphate ($CuSO_4$) were purchased from SRL, Sisco Research laboratories Pvt. Ltd., Mumbai. Folin-Ciocalteau phenol reagent, Potassium hydrogen phosphate (K_2HPO_4) and Potassium Dihydrogen Phosphate (KH_2PO_4) were purchased from S.D. fine chem. Ltd., Mumbai. Bromo phenol blue,acetic acid, agarose were purchased from Himedia, Mumbai. Glycerol was purchased from RANKEM Pvt Ltd. Pre stained molecular weight marker was purchased from Bio-Rad, Methanol,Silvernitrate,Sodiumthiosulphate were purchased from Nice chemicals Pvt.Ltd. India.Ethanol purchased from Trimurty Chemicals, India.

5.3 Preparation of MaltamylSepharose affinity matrix

8g of Sepharose 4B was suspended in 12 ml distill water. Sepharose 4B washed with PBS (pH 7.2) solution.Matrix was separated in powder form diluted with PBS solution.4g of maltamyl sepharose 4B matrix was washed with 6ml distill water.2.6 ml of 2N NaOH and 0.65 ml epichlorohydrin were added with 4g of maltamyl sepharose 4B.It was cover with aluminum foil and incubated at 40^0c for 2h.After incubation 100 ml of NaOH (8g) solution was added with maltamylsepharose 4B. It was flitered by watman filter paper and gel was washed with 500ml water.6ml of concentrated $NH_3$was added with 4g of Sepharose 4B and gel was again washed with distill water.Suction dried 4g Sepharose 4B was suspended in 3 ml of 0.2M K_2HPO_4 buffer, which was contain 51mg maltose .Suspension was incubated at room temperature for 10days.

5.4 Isolation and Purification of Con A

Con A. isolated from 50g of jack bean seed. Seed were soaked in 150ml PBS solution (pH 7.2) for overnight.Crushed in a mixture and total weight of seed past is 220.2g.Crude obtained from homogenous past after centrifuge at 7000 rpm for 20 min. at 4^0c.Collect the supernatant 50ml and added solid ammonia sulphate 8.2g to get 30% saturation. Stored 4^0 c for 12h. 30% saturationsample centrifuge at 7000 rpm at 4^0c for 20min.Collect the supernatant 45ml added ammonia sulphate 18.09g to get 90% saturation. Stored at 4^0 c for 12h.90% saturation sample centrifuge at 7000 rpm for 20 min at 4^0c.Pellet was collected and added 20 ml distill water, vortex the mixture properly.Then kept for dialysis in distill water for 2days and 2day in PBS (pH7.2). After dialysis protein sample 40ml was centrifuge at 7000 rpm for 20 min at 4^0c.Collect the supernatant and stored at 4^0c.

5.5 Affinity chromatography

Washed the maltamly sepharose beads in 700 ml PBS solution (pH7.2) and O.D was measured at 280nm. Protein sample 38ml was passed through maltamyle sepharose beads and its O.D was determined at 280nm.Maltamyle sepharose beads were again washed with 1000ml PBS solution (pH 7.2) and its O.D was measured at 280nm.50ml Maltose solution was loading on maltamly sepharose beads. And O.D was measured at 280nm of eluent.Kept 38ml of eluent for dialysis in 2lit PBS (pH 7.2) and stored at 4^0c for 1day.

5.6 Determination of protein concentration

The crude and 30% saturation sample was diluted 50 times by mixing 40µl of sample with 1960µl of PBS solution (pH 7.2).90% saturation sample was diluted 20 times by mixing 100µl with 1990µl of PBS solution (pH7.2).Sample obtained from affinity chromatography was directly taken for O.D without dilution. Their O.D was measured at 280 nm.

5.7 Estimationof protein by Lawry's method

REAGENT A-Sodium hydroxide(0.5%)

Sodium carbonate(2%) makes it upto 1 litre

REAGENT B1-1% Copper sulphate

REAGENT B2-2% Sodium potassium tartarate

REAGENT C-A: B1:B2-100:1:1

BSA STANDARD-1mg/ml

Folinciocalteau's reagent-1N (5 ml solution +5 ml distill water)

Take different concentration of BSA solution from stock solution and add distill water to it and made up to 2ml.Con A protein taken unknown quantity dissolved in 1ml distill water. And add reagent C of 5 ml and protein of 0.5ml. Mixed properly and incubate for 10 mins. Then 0.5 ml of Folin reagent was added and incubates for 30min. Take OD at 750nm.

5.8 Haemagglutination assay

1ml blood was collected in the presence of anticoagulation. Centrifuged at 1000 rpm for 5min at room temperature. Collect the pellet and added 10 ml of PBS, centrifuge at 1000 rpm for 5min at room temperature. Pellets were collected; take 100 µl from it and added 10ml of PBS solution (pH 7.2) Human erythrocyte suspension (10^6cells/ml) of blood was used for the Haemagglutination assay. The assay was carried out in a 96 well round bottom microtitre plate. Concanavalin A solutions were serially diluted (double dilution) and 100 µl volume of normalized sample and 100 µl of blood. Last well serve as negative since it contain 100 µl of blood and 100µl of PBS solution. The plates were incubated at room temperature for 2 h.

5.9 Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970)

Polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970).Stacking gels(5%) and resolving gels(12%) were prepared . The electrode buffer (pH 8.3) contained 0.025 M Tris, 0.192 M glycine, and 0.1% SDS. Gel loading buffer (5x) contained 50 Mm Tris-Cl (pH6.8), 100 mM β-mercapto ethanol, 2% (w/v) SDS. 0.1% bromophenol blue, 10% (v/v) glycerol. Proteins in the sample buffer were boiled for 3 min before electrophoresis. Electrophoresis was carried out until the bromphenol blue marker reached the bottom of the gel.The stacking gel was run at 90v and resolving gel was at 140 V.

5.10 Silver staining

Prepared fixing solution (75 ml) 37.5 ml methanol, 9 ml acetic acid, 37.5 µl formaldehyde and rest distill water and gel was stored in fixing solution for overnight. Washed with ethanol(200 ml) 3times in the interval of 15min. Gel was pretreatment with 60ml sodium thiosulphate solution for 1min. then again washed with double distill water 3times in the interval of 20 sec. Prepared the impregnation solution (0.12g silver nitrate, 40 µl formaldehyde and rest was distill water)and rinse with distill water 2 times in 20sec interval. Then develop the bands by using the solution of 36.6 g sodium carbohydrate, 2 crystal of sodium thiosulphate, 30 µl formaldehyde and rest was distill water, shacked it in a dark room till bands were developed for 15mins. Rinse in water 3 times in 20 second intervals. Finally gel was transferred into the stop solution. We used the fixing solution as stopping solution.

RESULT AND DISCUSSION

6. RESULT AND DISCUSSION

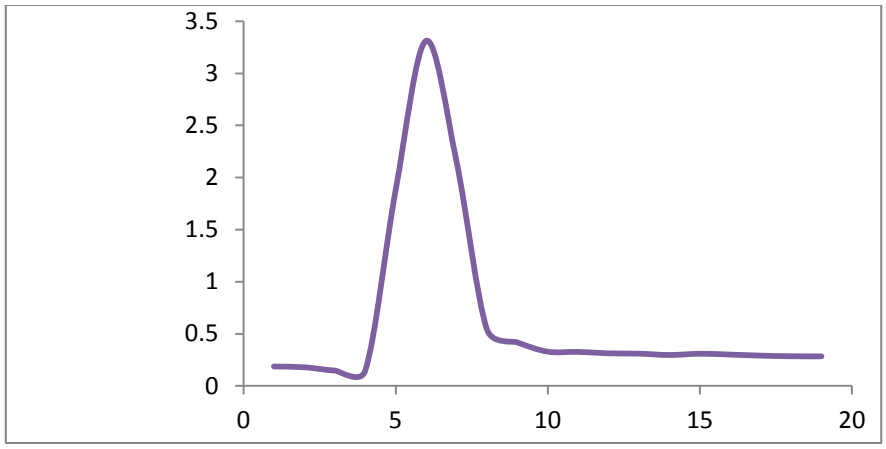
6.1 Purification of Concanavalin A

Jack bean extract was precipitated 30% to 90% by ammonia sulphate fraction method. It wasdialysed for 3 days with water and PBS. Dialysed supernatant was loaded on maltamly sepharose4B in the method of affinity chromatography.The unbound proteins were drained out during the washing of maltamly beads with PBS solution at each 10minsinterval. Those proteins were binds with maltamly sepharose beads, containing metal ions(Ca^{2+} or Mg^{2+}ions). The solutions of eluted protein had bound on maltose sugar than maltamly beads. Maltose was remove from the protein by the method of dialysis in PBS solution(pH7.2) for 1day. Hence purified protein was obtained.During the process of affinity chromatography we found 38 ml of eluentfor dialysis.

7(a)

7(b)

Fig-7(a) and (b)-Affinity chromatography and dialysis in water.



Graph-1: Elution profile of concanavalin A lectin from maltamylsepharose affinity matrix.

6.2 HemagglutinationAssay

The agglutination activity of the normalized sample of Jackbean was tested with the human RBC. The purified protein obtained by maltamylsepharose 4B beads containing Ca^{2+} ion and affinity purified protein exhibit a higher titer (2^2) with respect to crude ,30%and 90% dialysate portion .Hence the purified form of protein is a lectins and it is probably Con A.

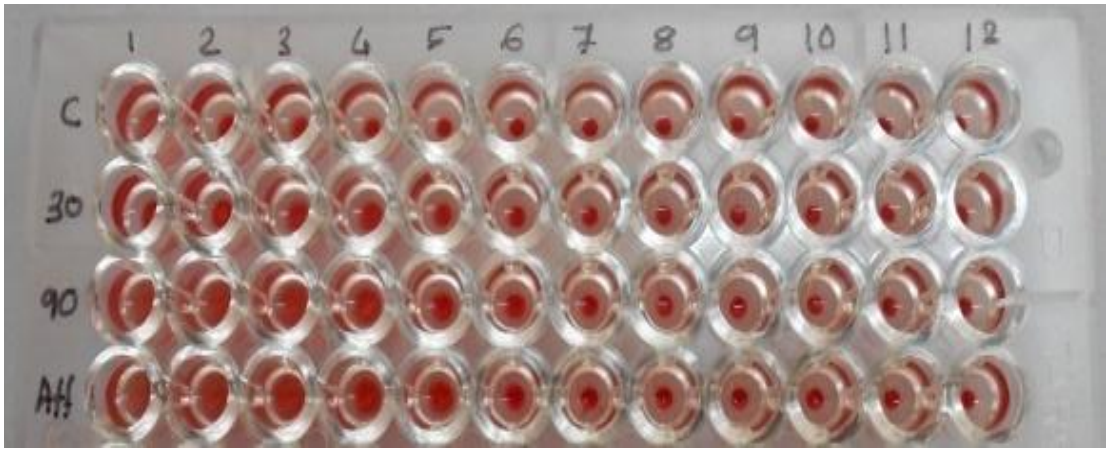


Fig 8 – upper panel of 96 well plate.

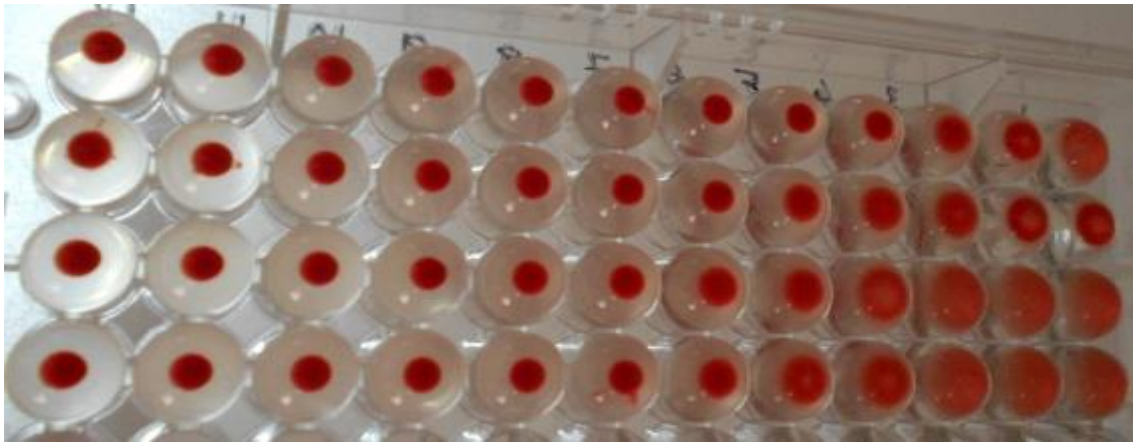


Fig 9- lower panel of 96 well plate.

6.3 SDS PAGE

Concanavalin A lectins purified from sephrose-4B beads runs into the SDS PAGE band was observed after silverstaining.Lane1 to 5 represent the crude, 30% cut, 90%cut, affinity and pertained molecular weight marker.There is a clear distinct band corresponding to 104 kDa in the affinity portion, this size corresponds to our protein of interest (ConA).

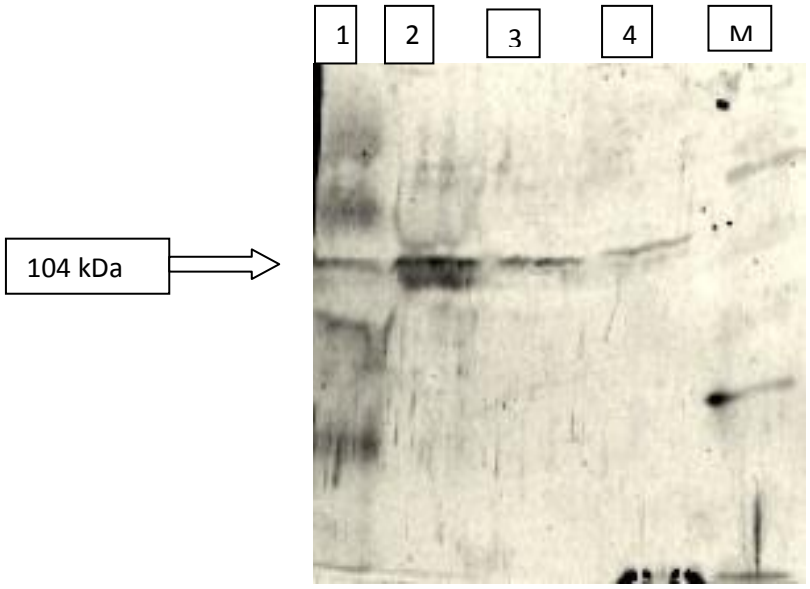


Fig- SDS PAGE

CONCLUSION AND FUTURE PROSPECT

7. CONCLUSION

Concanavalin A was purified from jack bean (*canavalia ensiformis*) seed. From affinity chromatography bounded proteins are collected.Con A aggregation used to study for haemagglution activity of human RBC. The activity of agglutination exhibit higher titer (2^2) with respect to crude, 30%, 90% saturation and affinity portion. In SDS PAGE clearly distinct band corresponding to 104 kDa in the affinity portion. From the experiment we confirmed that our isolated lectins were concanavalin A.

8. FUTURE PROSPECT

The purified lectins are identically known as Con A. from the molecular weight 104 kDa in the process of SDS PAGE.Con A aggregation used to study for relationship between cell toxicity and aggregation process.It can be used as therapeutic drug for future references.It cross-links cell surface glycoproteins,thereby initiating various cellular responses, includingT-cell activation and triggerapoptosis pathway in cancer cells.The cost purified Con A by sepharose 4B beads is the support as compare to cost of sephadex G-50.

REFERENCES

9. REFERENCES

1. Van Rhijn P, Goldberg R B, Hirsch A M. *Lotus corniculatus* nodulation specificity is changed by the presence of a soybean lectin gene. Plant Cell1998; 10:1233–1250.
2. Skvortso I M, Ignatov V V. Extracellular polysaccharides and polysaccharide-containing biopolymers from Azospirillum species: Properties and the possible role in interaction with plant roots. FEMS Microbiol. Lett 1998; 165:223–229.
3. Rao K V, Rathore K S, Hodges T K, Fu X, Stoger E, Sudhakar D, Williams S, Christou P, Bharathi M, Bown D P, Powell K S, Spence J, Gatehouse A M, Gatehouse J A. Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. Plant J 1998; 15:469–477.
4. Van Parijs, J. Broekaet W.F, Glodstein I.J and Peumans, W.j , (heveabrasiliensis) an antifungal protein from rubber-tree latex, planta 1991; 183:258-262.
5. Van Damme, E.j.M, Brike,F.,Winter, H.C.,Van Leuven, F., Goldstein,I.J and Peumans, W.J. .Molecular cloning of 2 different mannose- binding lectins from tulips bulbs. European journal of Biochemistry 1996; 236:419-427.
6. Shu-Ye Jiang, Zhigang Ma, SrinivasanRamachandran , research article Evolutionary history and stress regulation of the lectin superfamily in higher plants, BMC Evolutionary Biology 2010;12:110-115.
7. Firon, N., S. Ashkenazi, D. Mirelman, I. Ofek, and N. Sharon, Hanski, ***E***., PA Horwitz, and MG Caparon. Microbial cell surface hydrophobicity: History, measurement, and significance, p. 361-386. In RJ Doyle 1990; 120:235-249.
8. Sumner JB, Gralën N, Eriksson-Quensel IB "The molecular weights of canavalin, concanavalin A, and Concanavalin B". The Journal of Biological Chemistry 1938; 125: 45–48.

9. V. A. Hilder, K. S. Powell, A. M. R. Gatehouse, J. A. Gatehouse, L. N. Gatehouse, Y. Shi, W. D. O. Hamilton, A. Merryweather, C. A. Newell and J. C. Timans, "Expression of snowdrop lectin in transgenic tobacco plants results in added protection against aphids" 1995;58:67-70.
10. Hyung-i Lee$, Willem F. BroekaertQll, and Natasha V. RaikhelSII From the $.Department of Energy, Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824-1312 and the SF. A .Janssens Laboratory of Genetics, Catholic University of Leuuen, W 1993;125:437-440.
11. Dipak K. Mandal, Nand Kishore, C. Fred Brewer Thermodynamics of Lectin-Carbohydrate Interactions. Titration Microcalorimetry Measurements of the Binding of N-Linked Carbohydrates and Ovalbumin to Concanavalin A 1983; 41:271-275.
12. Loris R, Hamelryck T, Bouckaert J, Wyns L."Legume lectin structure". Biochim. Biophys. *Acta* 1998; 1383: 9–36.
13. Becker, J., Reeke, G., Wang, J., Cunningham, B., and Edelman, G.: The Covalent and Three-Dimensional Structure of Concanavalin A. III. Structure of the Monomer and its Interaction with Metals and Saccharides, J BiolChem 1975; 250: 1513.
14. Goldstein, I., Hollerman, C., and Smith, E.: Protein-Carbohydrate Interaction. II. Inhibition Studies on the Interaction of Concanavalin A with Polysaccharides , Biochem1965;876:65.
15. Min W, Dunn AJ, Jones DH . "Non-glycosylated recombinant pro-concanavalin A is active without polypeptide cleavage 1992; 535:40-42.
16. Kakizoe T, Komatsu H, Niijima J, Kawachi T, Sugimura T. "Increased agglutinability of bladder cells by concanavalinA after administration of carcinogens"1980. Cancer Res 2009; 40: 200–201.
17. Becker FF, Shurgin A. "Concanavalin A Agglutination of Cells from Primary Hepatocellular Carcinomas and Hepatic Nodules Induced by N-2-Fluorenylacetamide" (PDF).Cancer Res 1975; 35: 2879.

18. Betton G R."Agglutination reactions of spontaneous canine tumourcells,induced by concanavalin A, demonstrated by an isotopic assay". Int J Cancer 1976; 18: 687–696.

19. Inbar M, Ben-bassat H, Sachs K. "A specific membrane activity on the surface membrane in malignant cell transformation". ProcNatlAcadSci, USA 1971; 68: 2748.

20. Sela B, Lis H, Sharon N, Sachs L."Quantitation of N-acetyl-D-galactosamine sites on the surface membrane of normal and transformed cells". Biochim .Biophys.Acta1971; 21:249- 564.

21.Gartner TK, Podleski TR ."Evidence that a membrane bound lectin mediates fusion of L6 myoblasts". BiochemBiophys Res Commun1975; 67: 972–973.

22.Weiser MM. "ConcanavalinA agglutination of intestinal cells from the human fetus". Science1976; 177: 525–6.

23.Cuatrecasas P."Interaction of wheat germ agglutinin and concanavalin A with isolated fat cells". Biochemistry1973; 12: 1312–1323.

24.Capo C, Garrouste F, Benoliel AM, Bongrand P, Ryter A, Bell GI. "Concanavalin-A-mediated thymocyte agglutination: a model for a quantitative study of cell adhesion". J CellSci1982; 56: 21–48.

25. Noonan KD, Burger MM. "The relationship of concanavalin a binding to lectin-initiated cell agglutination". J Cell Biol 1973; 59: 134–142.

26. Ofek I, Mirelman D, Sharon N. "Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors". Nature1977; 265: 623–625,

27. Doyle RJ, Birdsell DC."Interaction of concanavalin A with the cell wall of Bacillus subtilis". J Bacteriol1972; 109: 652–8.

28. Betton G R."Agglutination reactions of spontaneous canine tumour cells, induced by concanavalin a, demonstrated by an isotopic assay". Int J Cancer 1976; 18: 687–696.

29. Christine Wellsa, The Unit for Cancer Research, University of Leeds, Leeds U.K.T.C. Bøjg-Hansen The Protein Laboratory, University of Copenhagen Denmark, E.H. Coopera, M.R. Glass. The Department of Obstetrics and Gynaecology, University of Leeds, Leeds U.K. Received 1980;122:320-323.

30.BettonGR."Agglutination reactions of spontaneous canine tumourcells,induced by concanavalin a, demonstrated by an isotopic assay". Int J Cancer1976; 18: 687–696.

31.Verti, V.R Carrotta, P.Picone, M.D. Carlo and V.Militello, Concanavalin A aggregation and toxicity on cell culture. Biochem, Biophys 2010; 11:153-157.

32. LiuB,M.W.Min and J.K Bao.Induction of apoptosis by Concanavalin A. and its molecular mechanism in cancer cells Autophagy 2010;329:348-352.

33.H. Bashir, T. Khan, A. Masood and R.Hamid, isolation, purification and characterization of a lectins from a lock Kashmiri varity of soybean(glycine max) Asian journal of biochemistry 2010;5:145-153.

34. Mary J., Maliarik and Irwin.j. glodstein.”photoaffinity labeling of the Adenine Binding site of the lectins from lima bean, Phaseoluslunotus, and the kidney bean phaseolus vulgaris.
From the department of Michigar Ann Arbor, Michigan1983; 25:137-142.

35.Clara L. Díaz, Leo S. Melchers, Paul J. J. Hooykaas, Ben J. J. Lugtenberg & Jan W. Kijne,Root lectin as a determinant of host–plant specificity in the Rhizobium–legume symbiosis.Department of Plant Molecular Biology, Leiden University,Nature Structural Biology 1995;2: 472 – 479.

ABBREVIATION

PBS: Phosphate Buffer Saline

et al: And others

Rpm: Rotation Per minute.

Conc: Concentration

Hrs: Hours

L: litre

Mg: Milli gram

pH: Hydrogen concentration

NaOH: Sodium hydroxide

Na_2CO_3 : Sodium carbonate

APS: Ammonium per sulphate

TEMED: N,N,N',N'-tetramethylenediamine

$KNaC_4H_4O_6$: Potassium sodium tartarate

SDS-PAGE: Sodium Dodecyl sulphate Polyacrylamide Gel Electrophoresis.

BSA: Bovine serum albumin

KH_2PO_4: Potassium Dihydrogen Phosphate

K_2HPO_4: Potassium hydrogen phosphate

$(NH_4)_2SO_4$: Ammonium Sulphate

Pvt .Ltd: Private limited

kDa: Kilo Dalton